

Statistical mechanics of coil-hairpin transition in a single stranded DNA oligomer

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Abstract

A model of self-avoiding walk with suitable constraints on self-attraction is developed to describe the conformational behavior of a short RNA or a single stranded DNA molecule that forms hairpin structure and calculate the properties associated with coil-hairpin transition by enumerating all possible conformations of a chain of N monomers in two and three dimensions. The first and last five monomers of the chain have been allowed to pair and form the stem of the hairpin structure while the remaining monomers can form a loop. The coil-hairpin transition is found to be first order with large entropy change. While the rate of unzipping of the hairpin stem is found to be independent of the length of the loop and the dimensionality of the space, the rate of closing varies greatly with loop length and dimensionality of the space.

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The hairpin structure is often observed in RNA and single stranded DNA (ssDNA) molecules and is known to participate in biological functions such as the regulation of gene expression, DNA recombination, and facilitation of mutagenic events etc [1, 2, 3, 4, 5]. The stability and conformational fluctuations of the hairpin structure have recently been investigated by designing simple ssDNA oligonucleotides [6, 7, 8, 9, 10] that have few complementary bases at both ends of the chain and one type of nucleotides in the middle, *e.g.* 5'–**CCCAA** - $(X)_m$ - **TTGGG**–3' where X is any one of the four nucleotides and m its number. A hairpin structure has two structurally and dynamically distinct domains: a base paired stem and a single stranded loop connecting two halves of the stem. The stem shows the same response to change in solution conditions as a dsDNA oligomer. The loop region, however, shows a wide range of folding patterns that depend on the number and type of bases in the loop. The stem-loop structure fluctuates thermodynamically between different conformations, which in a simplified description, are divided into two main states: the open state when all the binding monomers are separated and the fully closed one where all the complementary bases are paired. However, as shown below, this simplification is not always correct.

The closed-to-open transition requires the large energy to unzip the base pairs of the stem whereas the closing transition requires the two arms of the loop come close to each other in space in such a way that hydrogen bonding interaction between the complementary nucleotides can take place. The understanding of the nature of the open-to-closed transition is essential to our understanding of biopolymer dynamics.

By attaching donor and accepter fluorophores to both ends of a ssDNA the open-to-closed conformational dynamics of the hairpin have been investigated [6, 7, 8, 9]. The fraction of the open state is displayed in terms of the melting curve, which depicts the variation of the static fluorescence intensity with temperature. The melting temperature T_m of the structure is defined as the temperature where the probabilities of the closed and open states are equal. In such experiments it is, however, difficult to tell whether the closed state corresponds to a hairpin structure with some base pairs intact [Fig. 1(a-c)] or whether it is merely a folded state where the donor and acceptor are quite close without base pairing [Fig. 1(d-f)].

Attempts have recently been made to understand the conformational fluctuations of hairpin-loop structure in telechelic chain using Monte Carlo simulations [11, 12]. In these calculations one or two attractive sites with spherically symmetric interactions have been

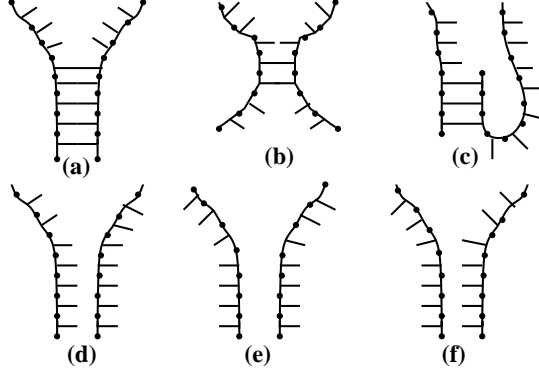


FIG. 1: Schematic representation of possible configurations of bases of the two ends of a chain on reaching close to each other. In (a) all the complementary bases are paired whereas in (b) and (c) the pairing is only partial. In situations shown in (d) to (f) pairing cannot take place even though two ends of the chain are close to each other.

attached at both ends of a chain of hard spheres. However, as stated above, in ssDNA and RNA the hairpin structure is formed due to pairing of complementary bases through hydrogen bonds which are highly directional and therefore, spherical nature of attraction taken in these studies does not represent the real situations.

In this communication we propose a lattice model of self-avoiding walk with constraints that mimic the condition of pairing of bases and calculate the conformational behaviour of the hairpin-loop structure. A self-avoiding walk of N steps ($N+1$ vertices) is considered on a square lattice in two-dimensions (2D) and on a cubic lattice in three dimensions (3D). A step of the walk represents a monomer of the chain. With each monomer a base is attached which has a direction associated with it. The first n bases of the walk represent nucleotide, say A (or C) and the last n bases the complementary nucleotide T (or G). The remaining $N - 2n = m$ bases represent one type of nucleotides which do not participate in pairing. The repulsion between monomers at short distances (*i.e* excluded volume) is taken into account by the condition of self-avoidance.

The pairing between bases can take place only when the bases at the two ends of the chain representing the complementary nucleotides approach on the neighbouring lattice bonds with their directions pointing to each other (see Fig. 1(a)). A base can at most pair with a complementary base. The base pairing can not take place when the two bases representing complementary nucleotides approach on neighbouring lattice bonds but with their directions

not pointing to each other as shown in Fig. 1(d-f). In Fig 1(b) and (c) a situation in which only partial pairing can take place is shown.

All possible conformations of ssDNA of N bases mapped by the self-avoiding walks [13] with the constraints specified above and having steps $N \leq 27$ on a square lattice (in 2D) and $N \leq 19$ on a cubic lattice (in 3D) have been exactly enumerated [14, 15]. The partition function of the system is found from the relation,

$$Z_N(T) = \sum_{i=0}^n C_N(i) (e^{-\epsilon/k_B T})^i \quad (1)$$

where $C_N(i)$ is the total number of configurations corresponding to walk of N steps with i number of intact base pairs. In all the results reported below we have taken $n = 5$ and $\epsilon = -0.08$ eV.

The partition function defined by Eq. (1) has six terms corresponding to six values of i from 0 to 5 ($i = 0$ corresponds to the open state, $1 \leq i \leq 4$ to the partially bound state and $i = 5$ to the fully bound state of the hairpin-loop structure). We can calculate the probability of these states for a chain of length N from the relation

$$P_i(T) = \frac{Z_N^i(T)}{Z_N(T)} \quad (2)$$

where $Z_N^i(T) = C_N(i) e^{-i\epsilon/k_B T}$ and $Z_N(T) = \sum_{i=0}^5 Z_N^i$.

Let the probability of the closed state which includes both partially and fully closed configurations of the chain is defined as

$$P_c(T) = \frac{Z_N^c(T)}{Z_N(T)} \quad (3)$$

where $Z_N^c(T) = \sum_{i=1}^5 Z_N^i$.

The values of $P_i(T)$ of partially bound states (*i.e.* for $1 \leq i \leq 4$) are found to be small compared to the values of $P_0(T)$ and $P_5(T)$ but not always negligible. Our results show that as the length of the part of the chain that forms loop in hairpin-loop structure increases, the values of $P_i(T)$ of the partially bound states decreases and is expected to be negligibly small when $m \gg 2n$. However, when $m \lesssim 2n$, the contribution due to partially bound states are significant and cannot be neglected. The values of $P_i(T)$ is found to peak at some temperature and the position of the peak shifts to the lower temperatures as i is increased from 1 to 4. The peak height decreases on increasing the chain length. In 2D for chains of

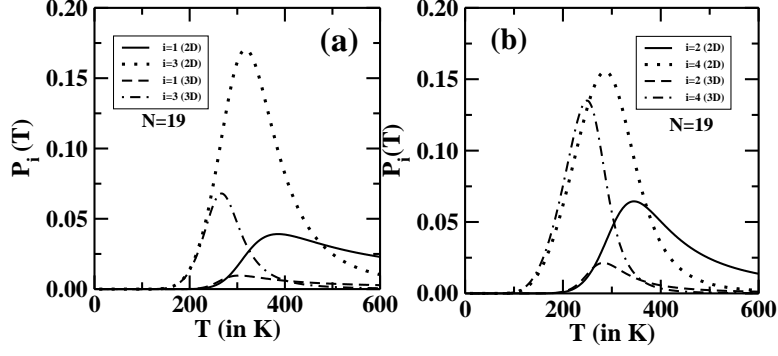


FIG. 2: Comparison of probability ($P_i(T)$) of partially closed states ($1 \leq i \leq 4$) for a chain of $N = 19$ monomers in two and three dimensions. (a) $i = 1, 3$ and (b) $i = 2, 4$.

small length, $N \leq 21$, the peak of $P_3(T)$ is found higher than that of $P_4(T)$ (see Fig. 2), but for $N \geq 23$ the peak of $P_4(T)$ becomes higher than that of $P_3(T)$. In 3D we, however, find that the peak of $P_4(T)$ is higher than that of $P_3(T)$ for all chain lengths studied by us. In Fig. 2, we plot the values of $P_i(T)$ found in 2D and 3D for a chain having $N = 19$ monomers to show the temperature dependence and magnitude of $P_i(T)$ of partially bound states. We note that both the peak height and the width of $P_i(T)$ are larger in 2D than in 3D. This shows that the probability of finding partially bound states is less in 3D than in 2D. This is because of the competition between thermal fluctuations which forces the chain to be in the open state and pairing of bases which take place when complimentary monomers approach each other in a particular way. Since the contribution arising due to thermal fluctuations in 3D is higher and the energy gained due to formation of pairs is same in 3D and 2D, the partially bound states are less stable in 3D than 2D.

The values of $P_0(T)$, $P_5(T)$ and $P_c(T)$ as a function of T are plotted in Fig. 3 for chains of $N = 15$ and 27 in 2D and in Fig. 4 for $N = 15$ and 19 in 3D. The difference in the values of $P_c(T)$ and $P_5(T)$ is due to partially bound states which are included in $P_c(T)$ but not in $P_5(T)$. The difference, as expected, seems to decrease on increasing the length of the chain and in going from 2D to 3D. The melting temperature T_m (*i.e.* the temperature at which transition from the hairpin-loop state to coil state takes place) is found from the intersect of $P_0(T)$ and $P_c(T)$ which takes place at their values of 0.5. We list in Table I the values of T_m for different chain lengths in both 2D and 3D cases. The value of T_m is found to have strong dependence on the chain length (T_m decreases as chain length increases) and on the dimensionality of the space.

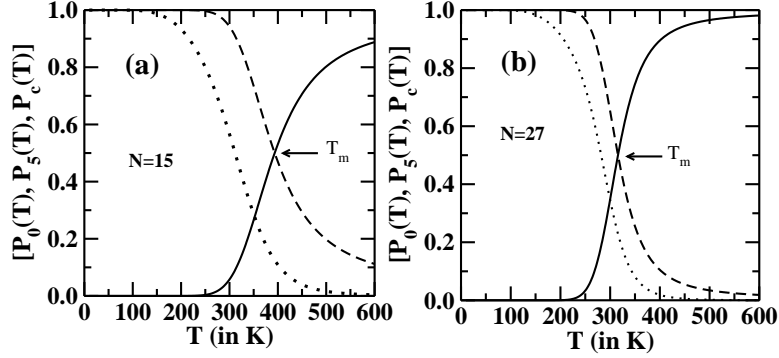


FIG. 3: Comparison of probability $P_i(T)$ of opened ($i = 0$) [solid line], completely closed ($i = 5$) [dotted line], and sum of partially and fully closed states ($\sum_{i=1}^5 i$) [dashed line] as a function of temperature T , in two dimensions. (a) $N=15$, (b) $N=27$.

The Helmholtz free energy and other thermodynamic quantities like the internal energy (U), the entropy (S) and the specific heat (C) can be found from the standard relations;

$$F = -k_B T \ln Z_N(T) = -k_B T (\ln Z_N^0 - \ln P_0(T)) \quad (4)$$

$$U = \sum_{i=1}^5 i \epsilon P_i(T) \quad (5)$$

$$S = - \left(\frac{\partial F}{\partial T} \right) = k_B \ln Z_N + \frac{U}{T} \quad (6)$$

$$C = -T \left(\frac{\partial^2 F}{\partial T^2} \right) \quad (7)$$

In general, the transition is located from the maxima of $C(T)$ curve. The temperature T_c at which maximum of specific heat is found, is lower than T_m . Using Eqs. (4) and (7) and the fact that Z_N^0 is temperature independent and $P_0(T_m) = \frac{1}{2}$ one can calculate the difference ($T_m - T_c$) for a given chain. We list in Table I the value of T_c for several values of N and note that as N increases T_c approaches to T_m and the peak in $C(T)$ curve becomes sharper. The calculated entropy shows a jump from low value corresponding to the hairpin structure to a value equal to $N \ln \mu$ (μ being the connectivity of the lattice for a SAW) at high temperatures.

The jump in entropy at T_m is found from the relation

$$\frac{\Delta S}{k_B} = - \frac{U(T_m)}{k_B T_m} \quad (8)$$

TABLE I: Values of transition temperature and change in entropy at the transition temperature T_m .

2D				3D			
N	T_c	T_m	$\frac{\Delta S}{k_B}$	N	T_c	T_m	$\frac{\Delta S}{k_B}$
	(in K)	(in K)			(in K)	(in K)	
15	346	393	3.82	13	337	359	5.02
19	323	352	4.71	15	300	311	6.32
23	310	330	5.33	17	282	392	6.93
27	300	315	5.91	19	271	279	7.37

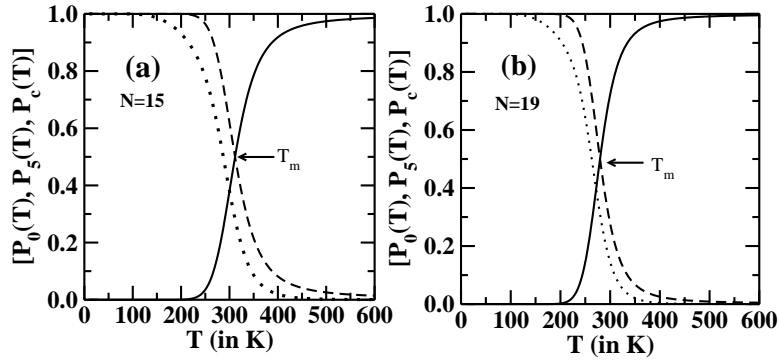


FIG. 4: Same as in Fig. 3, but in three dimensions and for (a) $N=15$ and (b) $N=19$.

We list the value of ΔS in Table I. The large change in entropy indicates that the transition is first order.

From the principle of detailed balance one gets

$$\frac{k_{o-c}}{k_{c-o}} = \frac{P_c}{P_o} \quad (9)$$

where k_{i-j} is the rate coefficient jumping from i to j state. The rate coefficient is assumed to follow the Arrhenius kinetics, $k_{i-j} = k_{i-j}^* \exp(-\beta \Delta F_{i-j})$, where ΔF_{i-j} denotes the free energy barrier associated with jumping from i to j states and k_{i-j}^* is a constant depending on the chemical nature of the chain. When the conformation changes from the open state to the closed one, the free energy barrier ΔF_{o-c} corresponds only to the entropy loss from a random coil to a ring polymer; and hence it should be temperature independent for a given

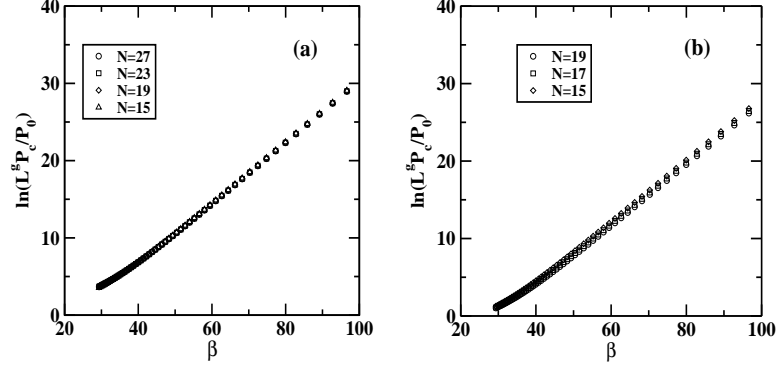


FIG. 5: $\ln(\frac{L^g P_c}{P_0})$ is plotted against β for several chain lengths N ; (a) in two dimensions with $L = m + 1$ and $g = 1.82$ and (b) in three dimensions with $L = m + 1$ and $g = 2.15$. Slope in both cases is equal to the internal energy of the fully bound state.

chain. On the other hand, when the structure fluctuates from the closed state to the open one, the energy barrier is the binding energy *i.e.* $\Delta F_{c-o} \simeq U$.

The probability that a polymer of length L folds on to itself to form a ring is given by

$$p_L = \frac{\sum_{i=1}^5 C_L(i)}{\sum_{i=0}^5 C_L(i)} \quad (10)$$

We find the main contribution to p_L is due to the term corresponding to formation of one pair. As suggested above k_{o-c} is proportional to p_L . For a self-avoiding walk of length L one finds [16]

$$p_L \propto L^{-g} \quad (11)$$

where $g = (d\nu + \gamma - 1)$. Here d , γ and ν are, respectively, the dimensionality of space, susceptibility and correlation length critical exponents. Substituting the value of γ and ν one finds $g = 1.83$ in 2D and 1.97 in 3D. From our results we find that $g = 1.82$ in 2D and $g = 2.15$ in 3D and $L = m + 1$. When we compare these values of g with those found from Eq. (11) a very good agreement is seen in 2D, but in 3D the value found by us is higher. However, the value of g found by Monte Carlo simulation on 3D lattice is 2.15 [17, 18] and the analytical estimates place its value in the range $2.10 \leq g \leq 2.20$ [19].

Using Eq. (9) we write

$$\frac{1}{k_{c-o}} = \frac{1}{k_{o-c}} \frac{P_c}{P_0} = \frac{1}{L^g k_{o-c}} \frac{L^g P_c}{P_0} \quad (12)$$

We plot in Fig. 5 $\ln(\frac{L^g P_c}{P_0})$ with $L = m + 1$ and $g = 1.82$ in 2D and 2.15 in 3D as a function of β for several chain lengths in 2D and 3D. As can be seen from these figures that the

values collapse into a single line with a slope equal to 0.4 ± 0.002 . The slope is same in both 2D and 3D cases and equal to the value of the internal energy of the fully closed state. This result indicates that regardless of the loop length and dimensionality of the space the thermal fluctuations provide a probability of $\exp(-\beta U)$ to unbind the closed conformation.

We have presented a of self-avoiding walk on a lattice with on-site repulsion and appropriate constraints on self-attraction to describe the conformational behaviour of a single stranded DNA and RNA molecules that form hairpin structure. For a given chain of N monomers, we have enumerated all possible configuration of the chain and using this data we calculated properties associated with the coil-hairpin transition. The transition is found to be first order with large entropy change. The transition temperature (T_m) is found from the intersection of the probability corresponding to the open state and the closed state which includes both partially and fully closed states. These probabilities are found to intersect at their values of 0.5. The transition temperature (T_m) decreases on increasing the loop length as has been observed in a real system [6]. The transition temperature, T_c found from the maximum of specific heat curve has the value lower than T_m and the gap between the two decreases as the length of the chain increases. The probability of the partially closed states are small and decreases on increasing the part of the chain that forms loop. The rate of unzipping of the hairpin stem was found to be proportional to $\exp(-\beta U)$ and independent of both the length of the loop and dimensionality of the space. The rate of closing is, however, found to depend rather strongly on both the loop length and the dimensionality of the space.

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